of the inserts in selected clones was determined by restriction digest analysis and confirmed by sequencing (Sequenase 2.0, USB). This procedure resulted in the isolation of the expression plasmids used, pPKR-AS (containing the PKR cDNA in an antisense orientation under the control of the CMV promoter in the vector) and p[Arg^{296}]PKR (containing the Arg^{296}PKR cDNA under the control of the CMV promoter in the vector).

Example 2: Isolation of PKR-deficient stable transfectants

[0047] Stable transfectants were obtained by electroporation of 5 X 10^6 exponentially growing U937 cells with 10 μg of each plasmid, in serum-free RPMI-1640 containing DEAE-dextran (50 μg/mL), with a Gene Pulser apparatus (BioRad) set at 500 μF, 250V. Bulk populations of stable transfectants were obtained by selection with 400 μg/mL geneticin (GIBCO-BRL) for 3 weeks. Clonal lines were subsequently obtained by limiting dilution cloning. Cell lines were cultured in RPMI-1640 containing 10% fetal calf serum (complete media) and geneticin.

[0048] Five representative cell lines were selected for initial characterization: "U937-neo" (also called U9K-C) was the control cell line transfected with the parental vector, pRC-CMV; "U937-AS1" (also called U9K-A1) and "U937-AS3" (also called U9K-A3) were independent clones transfected with pPKR-AS; "U937-M13" (also called U9K-M13) and "U937-M22" (also called U9K-M22) were independent clones transfected with p[Arg^{296}]PKR.

Example 3: Characterization of PKR-deficient transfectants

[0049] PKR kinase activity was measured in an autophosphorylation assay that uses poly(I):poly(C)-cellulose for binding and activation of PKR enzyme. PKR autophosphorylation assay was performed essentially as described by Maran et al. with the following modifications. Cell extracts (100 μg of protein per assay) were incubated with poly(I):poly(C)-cellulose for 1 hour on ice, washed three times, and incubated for 30 minutes at 30°C in 50 μl of a reaction buffer (20 mM HEPES (pH 7.5), 50 mM KCl, 5 mM 2-mercaptoethanol, 1.5 mM Magnesium acetate, 1.5 mM $MnCl_2$) containing 1 μCi of [γ-^{32}P]ATP. Proteins were separated on a 10% SDS- polyacrylamide gel and analyzed by autoradiography.

[0050] Cell extracts from IFN-treated HeLa and mouse L929 cells were used as positive controls, since PKR activity in these cells has been previously characterized (Meurs et al.) (FIG. 1A, lanes 1 and 8). U937-neo cells contained low basal levels of PKR activity which